2008

Variation in both male and female secondary sexual characters in the diving beetle, Agabus bipustulatus (L.) (Coleoptera: Dytiscidae)

Howe, W.

Howe, W. (2008) ' Variation in both male and female secondary sexual characters in the diving beetle, Agabus bipustulatus (L.) (Coleoptera: Dytiscidae)', The Plymouth Student Scientist, 1(2), pp. 56-86.
http://hdl.handle.net/10026.1/13811

The Plymouth Student Scientist
University of Plymouth

*The Plymouth Student Scientist, 2008, **1**, (2), 56-86*

Variation in both male and female secondary sexual characters in the diving beetle, *Agabus bipustulatus* (L.) (Coleoptera: Dytiscidae)

William James Howe

2008

Project Advisor: Dave Bilton School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA

Abstract

Sexual conflict and intersexual arms races occur due to the differing evolutionary interests between the sexes. Under the model of sexual conflict it is predicted that this will lead to sexual dimorphism, common in diving beetles, where males have suckers which they use to attach to the females' dorsal surface to potentially increase their copulatory ability. These are often met by female counteradaptations in an attempt to avoid multiple and potentially costly matings. This can result in a cyclical battle of responses in both sexes. Within the widely distributed species, *Agabus bipustulatus* there is known variation in female sculpture from intensely matt individuals that are highly reticulated to smooth, shiny individuals where reticulation is highly obsolete. We investigate this quantitatively across three populations that differ in reticulation and we also quantitatively investigate whether there are differences in male attachment devices. We show that reticulation of females varies markedly across the three populations of *A.bipustulatus* and suggest that the degree of reticulation in females may correspond to variation in male attachment devices. We suggest that in populations of highly reticulated females more small and large suckers are selected for in males to presumably aid their ability to coerce the female to mate.

Keywords: *Agabus* - Sexual conflict - evolutionary arms race – dimorphism-counteradaptation - reticulation.

ISSN 1754-2383 [Online]

Introduction

Sexual conflict arises when the reproductive interests of interacting males and females diverge, which can lead to evolutionary arms races between the sexes (Chapman *et al.*, 2003). In many organisms sexual conflict can arise as females invest substantially more energy in their offspring and therefore tend to be a lot more selective than males over their choice of mates (Thornhill & Alcock, 1983; Miller 2003). The differing evolutionary interests between the sexes are primarily caused by the costs of anisogamy and parental care (Bateman, 1948; Trivers, 1972; Yasui 1998).

For females copulation with one male is usually enough to fertilise all her eggs, however it is beneficial for males to mate with many females to maximise their reproductive success (Arnqvist, 1989a; 1997; Bergsten *et al.,* 2001). Although potentially beneficial in some aspects such as nuptial gifts (Vahed, 1998), and genetic benefits (Yasui, 1998), multiple mating can reduce the fitness of females and therefore they are expected to resist the male mating attempts, whilst males are expected to force copulation (Alexander *et al.,* 1997; Miller, 2003). The female's fitness can be reduced due to the energetic cost of reproduction and therefore reducing her foraging efficiency, increased risk of predation, risk of disease and parasite transmission and risk of death or injury inflicted by the male (Watson *et al.,* 1998; Daly, 1978; Parker, 1979; Arnqvist, 1989a). The asymmetric costs and benefits involved in mating can lead to sexual conflict and may fuel sexually antagonistic coevolution (Chapman & Partridge, 1996; Watson *et al.,* 1998; Arnqvist & Rowe, 2002). Miller (2003) suggested that males will evolve behaviours and morphologies that aid them in overcoming female resistance, and females will respond by evolving counteradaptations to the male's advantage, in a reciprocal sexual arms race.

Male adaptations to female resistance have been reported in a range of insects. Such as the grasping apparatus of the male water striders (Gerridae) (Arnqvist, 1989b), genital claspers in bush crickets (Sakaluk *et al.,* 1995) and anchored genitalia in seed beetles (Edvardsson & Tregenza, 2005). Evidence for female counteradaptations is rare in comparison to male adaptations to female resistance (Eberhard, 1985; Arnqvist & Rowe, 1995; Andersen, 1997; Miller,

ISSN 1754-2383 [Online]

2003). However the abdominal spines, unique to some female water striders, appear to have evolved to reduce the frequency of multiple matings, giving females more control over copulation (Arnqvist & Rowe, 1995).

Some species of diving beetles (Coleoptera: Dytiscidae) show clear examples of sexual dimorphism. To date most of the work on these modifications has been carried out on the subfamily Dytiscinae (Balfour-Browne, 1940; 1950; Bergsten *et al.,* 2001; Miller, 2003; Bergsten & Miller, 2007). It has been put forward that the mating behaviours and secondary sexual characteristics have resulted from an intersexual arms race as predicted by the model of sexual conflict (Bergsten, 1999; Bergsten *et al.,* 2001; Miller, 2003).

Males of the subfamily Dytiscinae have developed dilated tarsal segments on both their front and middle two legs, on which are attached modified adhesive setae or discs (Aiken & Khan, 1992; Bergsten *et al.,* 2001). Aiken (1992) observed the mating behaviour of *Dytiscus alaskanus,* and revealed that males strike their protarsi on the female's pronotum and elytra to grasp the female without any precopulatory courtship. Unique to diving beetles these mating behaviours can be costly for females as the male restricts the female from accessing atmospheric oxygen, which they depend on, resulting in the female becoming exhausted and nonresisting (Bergsten & Miller, 2007). Females often respond to male attachment by quick and erratic swimming in an attempt to dislodge the male, and avoid costly matings (Aiken, 1992; Miller, 2003).

In many species females are often dorsally structured which has been suggested to be counteradaptations to the males sucker pads (Bergsten *et al.,* 2001; Miller, 2003). Male diving beetles are usually smooth dorsally compared to some highly modified females such as *Dytiscus* species (see Miller, 2003). These modified females were considered by Darwin (1871), who suggested that the modifications acted as aids to improve male reproductive success. However sexual conflict has now been used to explain these modifications, and they actually reduce the ability of the suckers to attach to females (Bergsten *et al.,* 2001; Miller, 2003). Sexual conflict has resulted in a coevolutionary arms race in some diving beetles where counteradaptations such as female dorsal modification are matched by changes in the suction cups of males (Bergsten & Miller, 2007).

ISSN 1754-2383 [Online]

To date work on female sculpture and male secondary sexual characteristics has largely been carried out on the subfamily Dytiscinae. Quantitative studies on other subfamilies is limited to work by Bilton *et al.* (2008) who studied sexual dimorphism in *Hydroporous memnonius*. Sexual dimorphism is widespread in the subfamily Dytiscinae but here I present the first analysis of this in a member of the Agabinae- *Agabus bipustulatus (L).* Two distinct types of reticulation termed primary and secondary reticulation can be identified on the elytra and pronotum of *Agabus bipustulatus* (Figure 1). The small primary reticulation meshes resemble a honeycomb pattern embedded on the elytra and pronotum, imposed on which is the additional, larger secondary reticulation meshes (Jeannel, 1925; Balfour-Browne, 1950). This species has a wide distributional range and can be found over most of Europe and North Africa (Nilsson & Holmen 1995). Some populations which are narrower, more elongate and more dull have been called *Agabus solieri(A.)* (Balfour-Browne, 1950). The status of *A.solieri* is unclear as some argue that *A.solieri* is an independent species (Zimmermann, 1919; 1920; 1934; Falkenström, 1940) whereas others believe it is a variant of *A.bipustulatus* (Sharp, 1882; Balfour-Browne, 1950). More recent delimitation studies suggest that *A.solieri* is simply a conspecific, cold-adapted form of *A.bipustulatus* (Drotz *et al.,* 2001; Drotz, 2003). In terms of reticulation there is extreme variation in *A.solieri* species some of which are highly reticulated and others which have shining surfaces, where the females are male like (Sharp, 1882; Balfour-Browne, 1950). Some high altitude populations of *solieri,* called *Agabus kiesenwetterii (S.)*, have strongly shiny male like females where the reticulation is much wider and shorter than the typical temperate European species (Sharp, 1882; Balfour-Browne, 1950). However this has never been quantified so we include these here.

In this scanning electron microscope study of *Agabus bipustulatus,* populations from England, Spain and the high altitude *A. kiesenwetterii* from Serbia were selected that are known to differ in female microsculpture. Here we quantified primary and secondary reticulation in both sexes of all populations and also quantify the differences in male attachment devices (Figure 2) in all three populations.

ISSN 1754-2383 [Online]

Materials and Methods

Sample collection

Agabus bipustulatus specimens were collected using a D-framed pond net (30x25 cm; 1 mm mesh) from populations in England, Serbia and Spain (See table 1 for details of collected specimens). Beetles were killed using ethyl acetate vapour and were preserved in 70% ethanol until they were prepared for electron microscopy. Prior to preparation beetles were sexed using a light microscope, where males are distinguishable by the widened three basal segments of the tarsi on both the front and middle legs.

Preparation

Five males and five females from each population were prepared for scanning electron microscopy (SEM). After being dried specimens were mounted onto metal stubs, dorsal side up, allowing the reticulation of the elytra and the pronotum to be examined. In the case of males, left front and middle legs were removed and mounted alongside, to view the underside of tarsi. Beetles and appendages were attached to the metal stubs using a quick drying silver paint. An Emitech K550 sputter coater was used to coat the specimens in a fine layer of gold providing an electrically conductive film revealing surface topography of the sample.

Electron microscopy and image analysis

Specimens were photographed using a JEOL JSM5600 LV scanning electron microscope. The pronotum and elytra of both sexes were photographed at x 150 to quantify the secondary reticulation, and x 1000 for quantifying the primary reticulation. This resulted in four images of reticulation from each specimen examined. To ensure the same region of the elytra and pronotum were examined on each specimen the base of the pronotum or elytra was lined up in the corner of the SEM screen at x 50 and the magnification then increased appropriately. Male

ISSN 1754-2383 [Online]

tarsi were photographed at x 200. Two photographs were taken of both the front and middle legs. One which incorporates basal segments two and three and the other of the larger, basal segment one (Figure 2B). It was also important to ensure all sucker pads were at the same orientation prior to photography for accurate quantification.

All collected specimens (see table 1) were scored to establish the frequencies of beetles that have primary reticulation. To quantify structures of males and females, UTHSCA image tool 3.0 was used. The primary reticulation of the prepared specimens from each population was quantified. The area of ten individual primary reticulation polygons were measured (when present) on both the elytra and pronotum of males and females. For secondary reticulation in both males and females the length, width and area of ten secondary reticulation polygons were measured (from a transverse line across the image where the reticulation was length ways and at 90º to the line). This was carried out on both the elytra and the pronotum of each specimen.

With the males preliminary analysis revealed three different sizes of suckers; small, medium and large (Figure 2C). The small suckers were clearly a separate size class, and to confirm whether large and medium suckers were separate classes a two-sample *t*-test was used ($T=12.24$, $P<0.0001$, DF 21). The total numbers of large, medium and small suckers were counted and the areas of large and medium size suckers on the three basal tarsal segments were measured using the image analysis tool. The area of ten small suckers were measured from segment one for both front and middle legs. The maximum width of the three basal tarsal segments was also measured from both legs.

The length of photographed individuals was measured from the front of the pronotum to the apex of the elytra to avoid measurement error. This was carried out using a light microscope fitted with an eyepiece graticule, to distinguish whether beetles varied in size across the populations and between the sexes.

ISSN 1754-2383 [Online]

Data analysis

A combination of *t*-tests and one and two-way ANOVAs were used to assess variation between the three population in terms of area of female elytral and pronotal primary reticulation meshes, area and shape of elytral and pronotal secondary reticulation in both sexes, male small, medium and large sucker area between both legs and variation in beetle length between the three populations. Tukey's HSD test was employed after all one-way ANOVAs. When significant differences arose from two-way ANOVAs further one way ANOVAs employing Tukey's HSD test was used to determine where the differences lay.

Variation in tarsal width, large, medium, and small sucker number were investigated between the males of the three populations using nested repeated measures ANOVAs. Number of suckers on each segment, nested within foot and foot nested within beetle was analysed. Minitab 15 was used to complete all *t*-tests and one and two-way ANOVAs. Nested repeated measures ANOVAs were carried out in Statview 5.01.

Results

All English males have traces of primary reticulation on the elytra (Figure 5A) but this is lacking on the elytra of Serbian (Figure 5E) and Spanish males (Figure 5I). Primary reticulation is absent from the males pronotum (Figure 5B,F,J), except for a small proportion of Spanish individuals (see table 2). Where this reticulation is present on males it is usually very degraded resulting in a smooth and shiny dorsal surface compared to females. Percentages of the studied populations both male and female containing primary reticulation can be seen in table 2.

Female primary reticulation varies from being highly marked which is typical of English populations to highly obsolete such as the Serbian populations and the Spanish populations which are in between.

This study shows that females from England, Serbia and Spain differed significantly in the size of elytral primary reticulation ($F_{2,14}$ =15.62, P<0.001) (Figure 4). Tukey's HSD test revealed that the primary reticulation of the English

ISSN 1754-2383 [Online]

females was much smaller (mean area=46.56µm² ± SE 0.714) than the elytral primary reticulation of the Serbian females (mean area 75.36µm² ± SE 3.71) and Spanish females (mean area 70.68 µm² ± SE 5.62).

As well as being smaller the English female's primary reticulation (Figure 5C,D) appears to be more prominent and impressed than the Spanish populations (Figure 5K,L) resulting in a highly defined matt dorsal surface on English females. The elytral primary reticulation of the Serbian females appears to be highly degraded and are completely absent from the pronotum resulting in smooth and shiny dorsal surface (Figure 5G,H).

As stated above Serbian females lacked any pronotal primary reticulation. Pronotal primary reticulation was evident in English (mean area 40.57 µm² ± 3.23SE) and Spanish females (mean area 49.73 µm² ± 5.1ÕSE),however there was no significant difference in size between them (T=1.52, P= 0.19, DF=5). Pronotal primary reticulation is much more strongly impressed in the English females (Figure 5D) than the Spanish individuals where it its much weaker resulting in a smoother surface (Figure 5L).

Analysis shows that Serbian individuals differ in the size and shape of elytral and pronotal secondary reticulation compared to English and Spanish individuals. Serbian individuals have much shorter and wider secondary reticulation resulting in a larger surface area per polygon, especially on the elytra. Overall the area of elytral secondary reticulation polygons (Figure 6A) differed significantly between the three populations ($F_{2,29}$=4.25, P=0.026). Tukey's HSD test shows that Serbian individuals have significantly larger secondary reticulation (mean area= 10481 µm² ± 1683SE) than English individuals (mean area= 8760 µm² ± 132ÕSE) and Spanish individuals (mean area= 8617 µm² ± 1568SE). There is no significant difference between the sexes in area of the secondary reticulation ($F_{1,29}$=0.28, P=0.600).

The elytral secondary reticulation is significantly shorter (Figure 6B) in Serbian individuals (mean length= 301.76 µm ± 40.67SE) than English individuals (mean length=406.49 µm ± 71.03SE) and Spanish individuals (mean length 364.64 µm ± 64.6ÕSE) ($F_{2,29}$=9.54, P=0.001). Here the strongest effect is a population effect however there is a sex effect as well. Over all the two sexes differ significantly

ISSN 1754-2383 [Online]

($F_{1,29}$ =6.89, P=0.015) where females usually have slightly longer reticulation. The way the sexes differ is consistent across populations (sex x population interaction $F_{2,29}$=1.34, P=0.280).

The width of elytral secondary reticulation (Figure 6C) also differed significantly between the three populations ($F_{2,29}$=118.64, P< 0.001). Serbian individuals have much wider secondary reticulation (mean width=37.229 µm ± 3.2SE) than English (mean width= 21.5 µm ± 4.02SE) and Spanish Individuals (mean width= 23.66 µm ± 2.3SE). Again there was a sex effect ($F_{1,29}$=11.24, P=0.003) where males usually had wider secondary reticulation than the females.

As with the elytra, the area of the pronotal secondary reticulation (Figure 6D) of Serbian individuals was greater than the English and Spanish populations, however here the difference between the areas were not significant ($F_{2,29}$=1.00, P=0.382). As with the elytra, overall there was no significant difference in area of secondary reticulation between the sexes of different populations ($F_{1,29}$=0.44, P=0.514).

Similar to the elytra, the pronotal secondary reticulation of Serbian individuals have significantly shorter secondary reticulation than English and Spanish individuals ($F_{2,29}$=10.02, P=0.001) (Figure 6E). Overall there was no significant difference in length between the sexes ($F_{1,29}$=0.22, P=0.646).

The width of the pronotal secondary reticulation (Figure 6F) also differed significantly within the three population ($F_{2,29}$=80.12, P=<0.001). The secondary reticulation of Serbian individuals (mean width = 34.053 µm ± 3.637SE) was much wider than English (mean width =23.876 µm ± 1.78SE) and Spanish individuals (mean width= 22.652 µm ± 1.408SE). A sex effect was present ($F_{1,29}$=6.16, P=0.02) where usually males have wider reticulation than the females.

Male tarsal widths differed significantly across the populations (Figure 7). English males have much wider tarsi than Serbian and Spanish males (repeated measures ANOVA $F_{2,24}$=106.024, P<0.0001) (Figure 8B, F & J). Tarsal width also differed significantly between the front and middle legs ($F_{1,24}$=162.44, P<0.0001), where tarsal segments on the front leg are wider than the equivalent segment on

ISSN 1754-2383 [Online]

the middle leg. On both legs tarsal segment one is widest, followed by two and three ($F_{2,24}$=17.356, P<0.0001) which is consistent across all three populations.

Females have setae attached to their basal tarsal segments however these have not evolved into highly modified suckers like those of the males (Figure 3). Large suckers could be found on some or all of the male's three basal segments (Figure 8). Where present the suckers are attached at the apex of each segment in a row (Figure 2B). These suckers consist of a sucker plate attached to a round stalk which is attached to the tarsal segment. Overall the three populations differed significantly in the number of large suckers present ($F_{2,24}$=15.645, P=0.0005) (Figure 9A). English males have the most suckers (Figure 8A & C) and Spanish individuals have the least (Figure 8I & K), large suckers being usually lacking. Only two Spanish males had large suckers and where present they were usually singular. Serbian individuals have an intermediate number of large suckers. Large suckers are distributed differently across segments ($F_{2,24}$=18.262, P<0.0001) where they are attached in greatest number on segment one of both feet and less on segment three with an intermediate number on two (Figure 8). The way the suckers are distributed across segments did not differ significantly between the three populations (Segment x population interaction ($F_{4,24}$=2.539, P=0.066) neither did the way suckers were distributed across the segment and the two feet (foot x segment interaction $F_{2,24}$=0.968, P=0.394).

The area of the large sucker plates also differed significantly between populations ($F_{2,17}$=6.60, P=0.012) (Figure 10A). The sucker plates were much smaller in Serbian individuals (mean area= 2200.8 µm² ± 213.3SE) than those of the English (mean area= 2931.8 µm² ± 567.1SE) and the Spanish (mean area = 3086.9 µm² ± 521.1SE). Overall English individuals have most large suckers on both legs followed by the Serbians however the English suckers are significantly larger than the Serbians (T=2.96, P=0.02, DF 6). In Spanish populations only two individuals had large suckers and therefore were excluded from this test, yet where present these are relatively large.

Medium sized suckers could also be found attached to all three basal segments (Figure 8I). Overall the populations differed in number of medium sized suckers ($F_{2,24}$=9.673, P=0.003). Serbian individuals have far fewer medium sized suckers than the Spanish who have the most and English who have an intermediate

ISSN 1754-2383 [Online]

number (Figure 8 & 9B). Number of medium sized suckers did not differ significantly between foot ($F_{1,24}$=0.388, P=0.5452). Medium suckers are also distributed differently across segments ($F_{2,24}$=7.467, P=0.003). The way these suckers are distributed across segments differs between populations (Segment x population interaction ($F_{4,24}$=4.941, P=0.0047). English and Spanish individuals have most suckers attached to segment one followed by two and then three. Serbian individuals have very few medium sized suckers on both feet.

The area of intermediate sized suction cups did not differ significantly between the English (mean area= 1033.4 µm² ± 53.9SE) and Spanish individuals (mean area 1139.6 µm² ± 44.3SE) (T=-1.52, P= 0.152, DF 13). In the Serbian populations only one individual had medium sized suckers (mean area 832 µm² ± 106SE) and therefore was not included in analysis.

Small suckers were present on all three basal tarsal segments on both legs in all three populations (Figure 8). The populations differ significantly in number of small suckers ($F_{2,24}$=69.413, P<0.0001) (Figure 9C). Serbian males have very few small suckers (Figure 8F & H) compared to English which have the most (Figure 8B & D). This is far more marked on segment one on both feet. Front and back feet behave similarly because they did not differ significantly in number of small suckers ($F_{1,24}$=2.715, P=0.1253). Small suckers are distributed differently across segments ($F_{2,24}$=3078.2, P<0.0001) where most are attached to segment one and fewest on three relating to the size of the expanded tarsal segments. The way the sucker are distributed among segments differs within feet and this differs between the three populations ($F_{4,24}$=4.126, P=0.011). Here English have most suckers on segment one followed by the Spanish and then the Serbians with the least. This pattern is similar on segments two and three on both legs however the effect is much less marked.

The area of the small suckers also differed significantly between the populations ($F_{2,29}$=20.42, P<0.0001) (Figure 10B). Here English males have smaller suckers (mean area= 71.484 µm² ± 5.544SE) than the Serbians (mean are= 83.190 µm² ± 3.615SE) and the Spanish (mean area 78.662 µm² ± 2.313SE).

Throughout all three populations there is an over all trend that males have larger bodysizes than the females ($F_{1,29}$=18.91, P<0.0001). Within the populations

ISSN 1754-2383 [Online]

Spanish individuals appear to have consistently larger body sizes than English and Serbian individuals ($F_{2,29}$=16.67, P<0.0001).

Discussion

There is marked differentiation in primary reticulation between the three populations of *Agabus bipustulatus*. The dorsal surface of the English females appears overall matt due to the prominent polygonal structure of small reticulation meshes in contrast to the high altitude Serbian population where reticulation is highly obsolete. The reticulation, common to many Dytiscids (Balfour-Browne, 1940; 1950; Bilton *et al.,* 2008), when present is often more pronounced in females where it may act to interfere with the males ability to attach to the elytra and pronotum and therefore ability to copulate (Bergsten *et al.,* 2001; Miller 2003). It appears that there are two processes working here where primary reticulation is already or becoming suppressed in males and more intensified in females (Balfour-Browne, 1940). As with the reticulation of matt female *H. memnonius* populations (Bilton *et al.,* 2008), the tighter network of smaller and distinct reticulation in some populations of *A. bipustulatus* may have evolved as a response to a male advantage in a reproductive arms race of adaptations and counteradaptations (Miller, 2003). This theory of adaptations in an arms race is consistent with work by Arnqvist and Rowe (1995) who provided the first evidence of female counteradaptations where the female abdominal spines in water striders reduce the ability of the male to copulate. However it must be reiterated that both males and females can have primary and secondary reticulation and therefore this would have evolved primarily for another function, not just as a female adaptation to avoid mating.

It would appear that the high altitude Serbian females, those largely lacking primary reticulation, would endure more costly multiple matings due to the simple mechanics of male suckers (Aiken & Khan, 1992; Bergsten 2005). It has been shown that male grasping devices are effective on smooth dorsal surfaces where full contact is maintained (Bergsten, 2005; Bergsten & Miller, 2007). One possible explanation for the apparent loss of primary reticulation may be due to the order of coevolution in a continuous evolutionary arms race. This has been interpreted

ISSN 1754-2383 [Online]

by Bergsten and Miller (2007) where Bayesian analysis of a diving beetle phylogeny was used to test the theory of arms races. Here *Acilius kishii* (also a high altitude species) females were found to have secondarily lost their dorsal modifications while males did not lose their suckers (Bergsten & Miller, 2007). These results supported the theory that arms races can escalate and de-escalate (Arnqvist & Rowe, 2002; Bergsten & Miller 2007). Alternatively, Bilton *et al.* (2008) suggested that modification to the sculpture of female *Hydroporus* species may reduce the hydrodynamic performance. This trade off between hydrodynamic performance and degree of reticulation in females may also apply to members of Agabinae.

Thin and elongate secondary reticulation is common to all specimens examined. This reticulation encapsulates the tighter network of small primary reticulation found on English and Spanish females and may also contribute to the ability of females to avoid copulation. Quantitative analysis of the surface structures of the *A.bipustulatus* complex was limited to Falkenström (1940) who identified *A.solieri* and *A.bipustulatus* as separate species and argued that secondary reticulation variation is the same between the two forms (reviewed by Drotz *et al.,* 2001). However this study suggests that the elytral secondary reticulation in the high altitude form of *A.solieri* is consistently shorter and broader than the English and Spanish *A.bipustulatus* populations, also noted by Sharpe (1882), further adding to the smoothness of the dorsal surface in the Serbian individuals.

Recent phylogenetic analysis of MtDNA suggests that *A.solieri* and *A.bipustulatus* are one species as gene flow between the two forms does occur (Drotz, 2003). It appears that *A.solieri* have multiple origin and are conspecific cold-adapted forms of *A.bipustulatus* (Drotz *et al.,* 2001; Drotz, 2003). However the debate over the status of *A.solieri* continues since initial observations from Sharp (1882). Balfour-Browne (1950) suggested that variation between *A.bipustulatus* species may be stimulated by climate and edaphic conditions however stressed that other factors must control the variation as the degree of reticulation can vary even at high atitudes.

ISSN 1754-2383 [Online]

It is also evident that the gaps between the secondary reticulation in English and Spanish individuals are much wider which is likely to reduce the suction ability as full contact of suckers is prevented (Bergsten, 2005). However this pattern in gap size is also evident in males which may suggest that thinner gaps have evolved at high atitudes in *A.solieri* for other purposes.

As highlighted in the introduction copulation can be costly to females and as a result it is predicted that females will be more selective over mate decision and not mate many times (Thornhill & Alcock, 1983). The reticulation on the dorsal side of females allows them to be more choosy during male encounters therefore acting as a potential screening mechanism (Bilton *et al.,* 2008) where only fit males will be able to attach and remain attached long enough to fertilise her eggs (Bergsten & Miller, 2007). It is likely that the female's modified dorsal surfaces of English and Spanish populations have evolved as a coevolutionary response to overcome the male advantage as predicted by the model of sexual conflict (Miller, 2003).

The study suggests that the degree and intensity of female's reticulation is associated with changes in the size of male tarsi and the number of suckers attached. Males from all populations have widened basal tarsal segments compared to the females. However males from English female populations, those with most extensive reticulation, have consistently wider tarsal segments than other males. This is also evident in male *H. memnonius* species from matt female populations (Bilton *et al*., 2008). Male suckers are modified setae attached to the tarsal segment helping them to attain copulation (Bergsten *et al.,* 2001). Setae are also present on the tarsi of female *A. bipustulatus*, however are not modified into attachment devices suggesting that the modified setae of males have evolved for copulatory purposes.

Agabus bipustulatus females from English populations are highly reticulated compared to Spanish females and especially Serbian females which are male like. In the highly reticulated female population, the number and size of the large suckers in males appears to be increased. This is similar to male *Graphoderus zonatus verrucifer* species where the three largest suckers were found to increase in size as the proportion of matt females in the population increased

ISSN 1754-2383 [Online]

(Bergsten *et al.,* 2001). Therefore suggesting males with larger suckers will be advantaged as the likelihood of successful copulation with matt females will be increased. This pattern where large suction cups are favoured in males from dorsally modified female populations has also been recognised in *Acilius* species (Bergsten & Miller, 2007). These large suckers in male *Agabus bipustulatus* species are likely to have evolved in response to changes in the female's reticulation. Bergsten and Miller (2007) suggested that large sucker cups maximise the suction force as the leaking channels will be reduced. In *A. bipustulatus* larger cups will cover a greater area of the female's reticulation covering more gaps between the primary and secondary reticulation and therefore the reticulation will be less effective at reducing suction force.

Intermediate sized suckers are also found on the basal tarsal segments of males from all three populations. This size class of sucker is largely absent from the high altitude Serbian males where females lack reticulation. Presumably males are selected for that have larger suckers, which are effective on smooth surfaces (Bergsten & Miller, 2007). Spanish males have the most intermediate sized suckers attached to their tarsi. As noted earlier the reticulation of Spanish females is of a lesser degree than English females. Therefore selection pressure for large suckers may be lower in Spanish males in contrast to English males where the suction force needed to remain attached to the female is much greater. It has been suggested that in *Graphoderus* populations with highly granulate females, intermediate sized suckers in males will be selected against as they are disadvantageous (Bergsten *et al.,* 2001). This may apply here and explain why males from highly reticulate English female populations have consistently fewer intermediate size suckers.

All males from the three examined *A. bipustulatus* populations have small sucker setae attached to the widened tarsal segments on both front and middle legs. These are found in much greater abundances on the tarsi of English males, which have the largest tarsal segments, most notably on segment one. It has been suggested that smaller and more numerous suckers in males may be more effective during copulation for attachment to dorsally modified females (Nilsson, 1986; Aiken & Khan, 1992), as well as the largest sucker plates (Bergsten *et al.,* 2001). This may apply to *A. bipustulatus* where English males have consistently

ISSN 1754-2383 [Online]

more small suckers presumably to aid the attachment to highly reticulate females. The high altitude Serbian males have the least small suckers which may be associated with the highly degraded reticulation of the females. This pattern of more numerous small suckers of males in female population that are dorsally modified can be seen in *Graphoderus* (Bergsten *et al.,* 2001). Small suckers are likely to be advantageous for males, as well as large suckers, as they can attach between leaking channels therefore increasing suction and attachment time for the male (Bergsten & Miller, 2007). It is likely that female reticulation increases the leakage of water into the males sucker, reducing suction and attachment time (Bergsten, 2005; Bilton *et al.,* 2008). Bilton *et al.* (2008) noted that small suckers in male *H. memnonius* appear to be of similar size to an individual microreticulation polygon of the var. *castaneus* females and suggested that this may be a counteradaptation to female's reticulation, however they did not quantify this. In *A. bipustulatus* English females have the smallest primary reticulation polygons and this is matched by males having notably smaller suckers than the reticulation polygons of the females. These small suckers of the English males are also the smallest of all three male populations. Larger primary reticulation polygons on Spanish and, where present, Serbian females are closely matched to the size of the males suckers. This supports the idea that small suckers in males have been selected for to aid attachment to individual polygon meshes, totally avoiding leaking channels which reduce suction force (Bergsten & Miller, 2007; Bilton *et al.,* 2008).

As noted earlier there is a size difference between males and females and more importantly between the three populations where Spanish individuals are larger than English and Serbian individuals. From this it may be expected that Spanish individuals would have more and larger suckers due to their size alone. However the results show that the variation in sucker number is size independent and presumably other factors such as degree of female reticulation are driving the variation in male suckers. This pattern where body size is not linked to variation in sucker number and proportion of modified females can also be seen in populations of *Graphoderus* species (Bergsten *et al.,* 2001; Bergsten 2005).

This study shows that within the *Agabus bipustulatus* complex different female morphs exists ranging from smooth to highly reticulate. This study demonstrates

ISSN 1754-2383 [Online]

that the degree to which the females are reticulated appear to be associated with modifications in males. In populations of highly reticulate females more large and small suckers are selected for in males presumably aiding the ability to coerce the female to mate. Bergsten and Miller (2007) suggest that small and large suckers are more effective for attachment to dorsally modified females.

This study provides evidence to support previous work on other Dytiscids where female adaptations have evolved in response to male grasping devices in an evolutionary arms race of cyclical battles as predicted by the sexual conflict model (Miller, 2003). Further research investigating more *A.bipustulatus* populations and dimorphic claw shape within these populations (Balfour-Browne, 1940) would provide useful, allowing further examination of the degree of which sexual conflict is acting within *Agabus bipustulatus.*

Similar to *A. bipustulatus,* members of *Agabus melanarius* (A.) also show this pattern where males in populations of modified females have widened tarsi and attached suckers (Figure 11). However the differences between the two species are striking. Sequential research on other members of Agabinae would provide useful comparative evidence of intraspecific variation in secondary sexual characters.

Acknowledgements

I would like to thank David Bilton for supplying specimens used in the study, statistical advice and for his support throughout. I would also like to thank Peter Bond and Roy Moate for their advice and assistance during SEM work.

ISSN 1754-2383 [Online]

References

Aiken RB. 1992. The mating behaviour of a boreal water beetle, *Dytiscus alaskanus* (Coleoptera: Dytiscidae). *Ethology, Ecology and Evolution* **4:** 245-254.

Aiken RB, Khan A. 1992. The adhesisve strength of the palettes of males of a boreal water beetle, *Dytiscus alaskanus* J Balfour Browne (Coleoptera: Dytiscidae). *Canadian Journal of Zoology* **70:** 1321-1324.

Andersen NM 1997. A phylogenetic analysis of the evolution of the sexual dimorphism and mating systems in water striders (Hemiptera: Gerridae). *Biological Journal of the Linnean Society* **61**: 345-368.

Alexander RD, Marshall DC, Cooley JR. 1997. Evolutionary perspectives on insect mating. In: Choe JC, Crespi B, eds. *The Evolution of Mating Systems in Insects and Arachnids.* Cambridge: Cambridge University Press, 4-31.

Arnqvist G. 1989a. Multiple mating in a water strider: mutual benefits or intersexual conflict? *Animal Behaviour*. **38:** 749-756.

Arnqvist G. 1989b. Sexual selection in a water strider: the function, mechanism and selection and heritability of a male grasping apparatus. *Oikos* **56:** 344-350.

Arnqvist G. 1997. The evolution of the water strider mating systems: causes and consequences of sexual conflicts. In: Choe JC, Crespi BJ, eds. *The Evolution of Mating Systems in Insects and Arachnids.* Cambridge: Cambridge University Press, 146-163.

Arnqvist G, Rowe L. 1995. Sexual conflict and arms races between the sexes: a morphological adaptation for control of mating in a female insect. *Proceeding of the Royal Society of London* **261:** 123-127.

Arnqvist G, Rowe L. 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature* **415:** 787-789.

Balfour-Browne WAF. 1940. *British water beetles* Volume 1 London: Ray Society.

Balfour-Browne WAF. 1950. *British water beetles* Volume 2 London: Ray Society.

Bateman AJ. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349-368.

Bergsten J. 1999. *Dytiscus* phylogeny and the evolution of secondary sexual characters (Coleoptera: Dytiscidae). Thesis Department of Undergraduate studies in Biology, University of Umeå: Umeå 43.

ISSN 1754-2383 [Online]

Bergsten J. 2005. Taxonomy, phylogeny and secondary sexual character evolution in diving beetles, focusing on the genus Acilius. Doctoral Dissertation. Department of Ecology and Environmental Science, University of Umeå: Umeå.

Bergsten J, Miller KB. 2007. Phylogeny of diving beetles reveals a coevolutionary arms race between the sexes. PLoS ONE 2 (6): e522.

Bergsten J, Toyra A, Nilsson AN. 2001. Intraspecific variation and intersexual correlation in secondary sexual characters of three diving beetles (Coleoptera: Dytiscidae). *Biological Journal of the Linnean Society*. **73:** 221-232.

Bilton DT, Thompson A, Foster G. 2008. Inter- and intra-sexual dimorphism in the diving beetle *Hydroporous memnonius* Nicolai (Coleoptera: Dytiscidae). *Biological Journal of the Linnean Society* in press.

Chapman T, Partridge L. 1996. Sexual conflict as fuel for evolution. *Nature* **381:** 189-190.

Chapman T, Arnqvist G, Bangham J, Rowe L. 2003. Sexual conflict. *Trends in Ecology and Evolution*. **18:** 41-47.

Daly M. 1978. The cost of mating. *American Naturalist*, **112:** 771-774.

Darwin CR. 1871. *The Decent of Man and Selection in Relation to Sex.* London: John Murray.

Drotz MK. 2003. Population genetics and phylogeography of the West Paleartic *Agabus bipustulatus* diving beetle complex. Doctoral Dissertation. Department of Molecular Biology, University of Umeå: Umeå.

Drotz MK, Nilsson AN, Saura A. 2001. The species delimitation problem applied to the *Agabus bipustulatus* complex (coleoptera Dytiscidae) in north Scandinavia. *Biological Journal of the Linnean Society.* **73:** 11-22.

Eberhard WG. 1985. *Sexual Selection and Animal Genitalia.* Cambridge, MA: Harvard University Press.

Edvardsson M, Tregenza T. 2005. Why do male *Callosobruchus maculatus* harm their mates? *Behavioural Ecology* 16: 788-793.

Falkenström G. 1940. Beitrag zur festen Begründung einiger Dytisciden- Arten, bzw. Zur Absonderung aus dem System anderer dergleichen, unter denen mehrere nur Kreuzungs-Produkte darstellen. *Arkiv för zoologi* **32:** 1-47.

Jeanell R. 1925. Morphologie de l'Elytre des Coléopères adephages. Archive *Zoologie Experimentale et General* **54**: 1-84.

ISSN 1754-2383 [Online]

Miller KB. 2003. The phylogeny of diving beetles (Coleoptera: Dytiscidae) and the evolution of sexual conflict. *Biological Journal of the Linnean Society*. **79:** 359-388.

Nilsson AN. 1986. Geographic variation in *Graphoderus zonatus* (Coleoptera: Dytiscidae) in Sweden. *Entomologica Scandinavia* **32**: 119-125.

Nilsson AN, Holmen M. 1995. The aquatic Adephaga (Coleoptera) of Fennoscandia and Denmark. II. Dytiscidae. *Fauna Entomologica Scandinavica* **32**: 1-192.

Parker GA. 1979. Sexual selection and sexual conflict. In: Blum MS, Blum NA, eds. *Sexual Selection and Reproductive Competition in Insects.* New York: Acedemic Press, 123-166.

Sakaluk SL, Bangert PJ, Eggert A-K, Gack C, Swanson LV. 1995. The gin trap as a device facilitating coercive mating in sagebush crickets. *Proceedings of the Royal Society of London.* B. **261:** 65-71.

Sharp D. 1882. On aquatic carnivorous Coleoptera or Dytiscidae. *The Scientific Transactions of the Royal Dublin Society Ser. 2*. **2:** 17-1003.

Thornhill R, Alcock J. 1983. *The Evolution of Insect Mating Systems*. Cambridge: Harvard University Press.

Trivers RL. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Decent of Man* (ed. Cambell,B), pp136-179. Heinmann educational.

Vahed K 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biological reviews* **78**: 43-78.

Watson PJ, Arnqvist G, Stallmann RR. 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. *American Naturalist*. **151:** 46-58.

Yasui Y. 1998. The 'Genetic benefits' of female multiple mating reconsidered. *Trends in Ecology and Evolution* **13**: 246-250.

Zimmermann A. 1919. Die Schwimmkäfer des Deutschen entom. Mus. Zur Berlin-Dahlem. *Archive für Naturgeschichte* **83:** 107-249.

Zimmerman A. 1920. Dytiscidae, Haliplidae, Hygrobiidae, Amphizoidae. In: Junk W, Schenkling S, eds. *Coleopterorum Catalogus* 1-326.

Zimmermann A. 1934. Monographie der paläarktischen Dytiscidae. V. Colymbetinae (1. Teil). *Koleopterologische Rundschau* **20:** 138-214.

ISSN 1754-2383 [Online]

Figure legends

Figure 1. (A) Elytral secondary & (B) primary reticulation. (Spanish *A.bipustulatus* female).

Figure 2. Male secondary sexual characters studied. A) underside of a middle leg of a Spanish *A.bipustulatus*. B) segments of tarsi examined (Serbian males front leg). C) the three different size classes of suckers examined throughout the study (Front leg of a Spanish *A.bipustulatus)*.

Figure 3. Basal segment of an English female *A. bipustulatus* front tarsus.

Figure 4. Female primary reticulation size across all three populations. (Grey= Elytra, White= Pronotum).

Figure 5. *Agabus bipustulatus* reticulation. A) English male elytra B) English male pronotum, C)English female elytra D) English female pronotum. E) Serbian (Solieri) male elytra F) Serbian (Solieri) male pronotum, G) Serbian (Solieri) female elytra, H) Serbian (Solieri) female pronotum. I) Spanish male elytra, J) Spanish male pronotum, K) Spanish female elytra & L) Spanish female pronotum.

Figure 6. Secondary reticulation size. Males (grey) and females (white). A), B) & C) length, width and area of elytral reticulation, respectively. D), E) & F) length, width and area of pronotal reticulation, respectively.

Figure 7. Male tarsal width of *Agabus bipustulatus.* (Grey= English populations, Grid= Serbian populations & White= Spanish populations). Histograms show mean values □SE. F= foot number and S= segment number.

Figure 8. Male tarsi and attached suckers
A),B),C) & D)English male tarsi. A) & B) Foreleg. C) & D) Middle leg.
E), F),G) & H) Serbian male tarsi. E) & F) Foreleg. G) & H) Middle leg.
I), J), K), & L) Spanish male tarsi. I) & J) Foreleg. K) & L) Middle leg.

Figure 9. Total number of male tarsal suckers in *Agabus bipustulatus.* (Grey= English population, Grid= Serbian population & white= Spanish populations. A) Large suckers; B) medium sized suckers & C) small sucker. Histograms show mean values □SE. F= foot number and S= segment number.

Figure 10. Male tarsal sucker area. (Grey= Foreleg, White= middle leg). A) Large suckers; B) small suckers. Histograms show mean values □SE. F= foot number and S= segment number.

Figure 11. *Agabus melanarius.* A) Female secondary reticulation (Elytra). Notice the reticulation is much shorter and broader than *Agabus bipustulatus* species. B) Female Primary reticulation (Elytra). Notice the reticulation is very degraded compared to English *Agabus bipustulatus* species. C) Male tarsi and attached suckers (Front leg). D) Male tarsi and attached suckers (Middle leg). Notice the tarsi are widened but not to the extent of *Agabus bipustulatus.* There are far fewer suckers and those present appear to be much smaller compared to *Agabus bipustulatus* species.

ISSN 1754-2383 [Online]

Figure 1

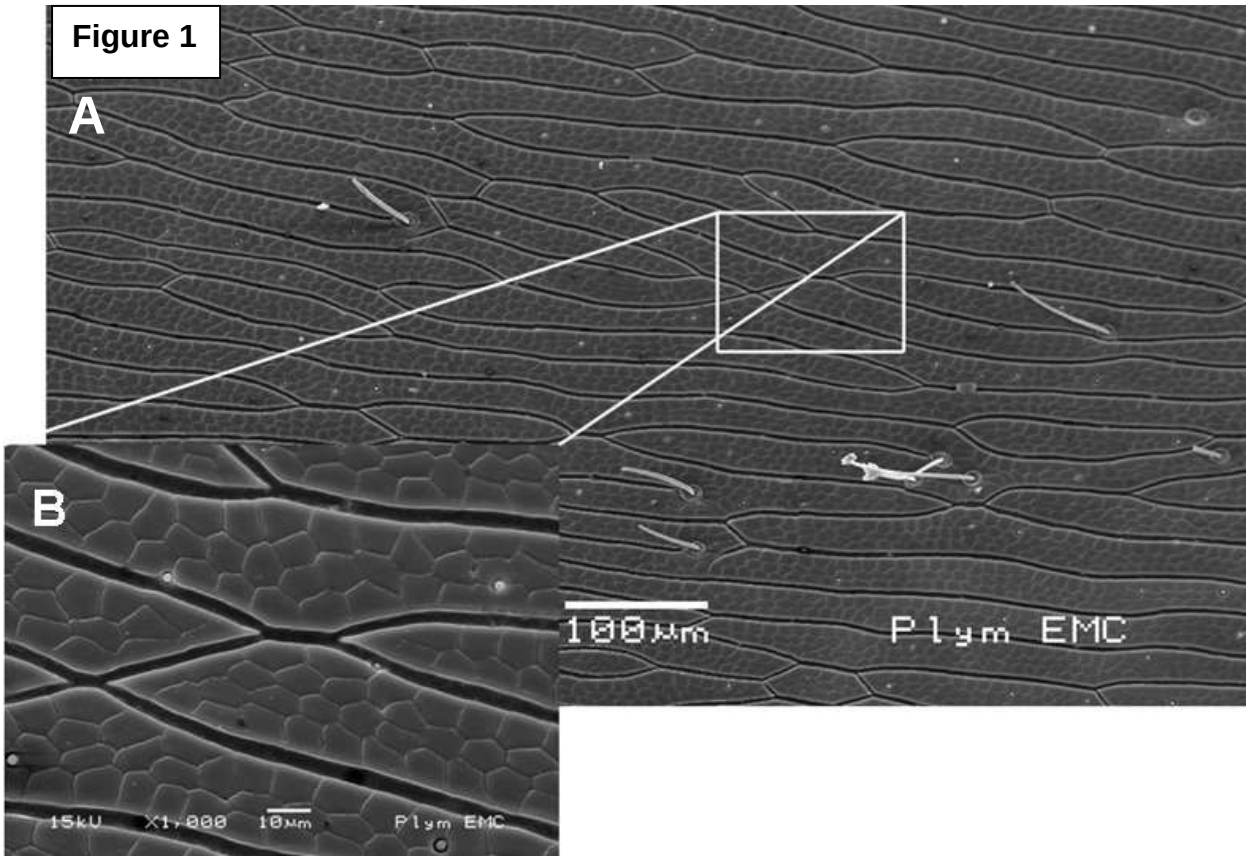
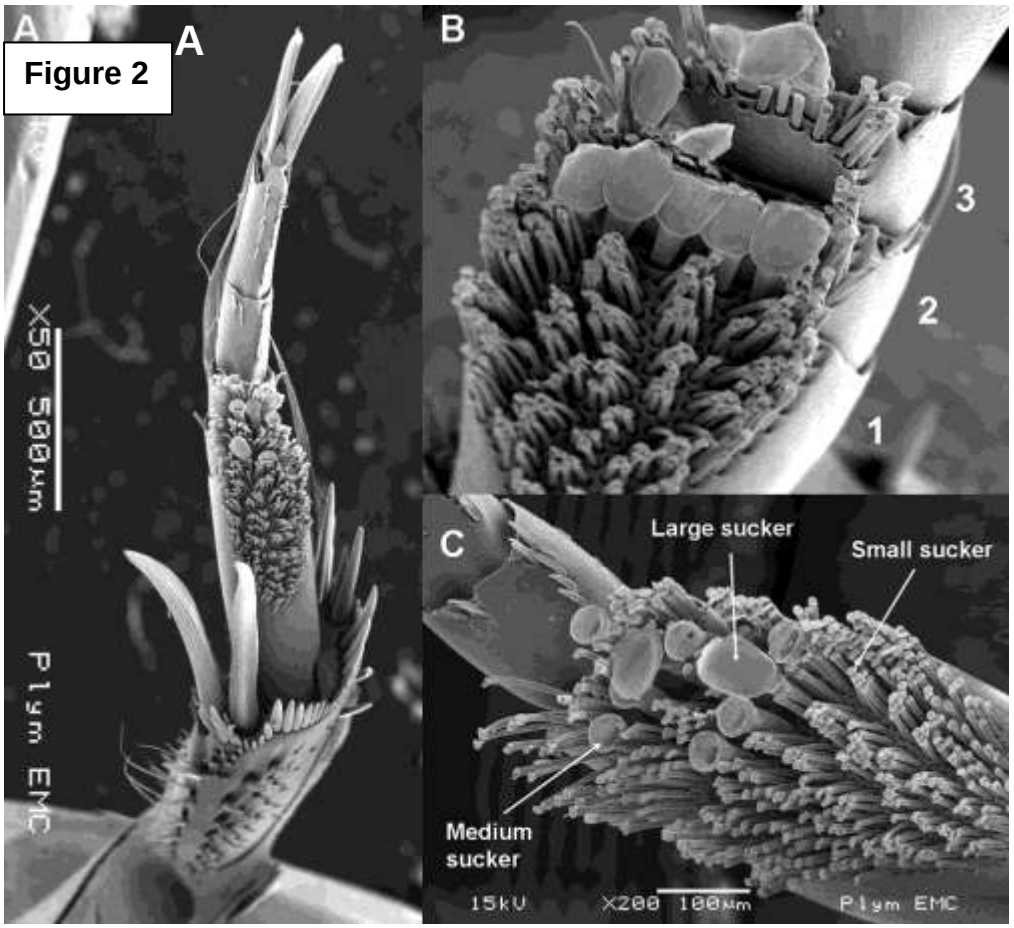


Figure 2



ISSN 1754-2383 [Online]

Figure 3

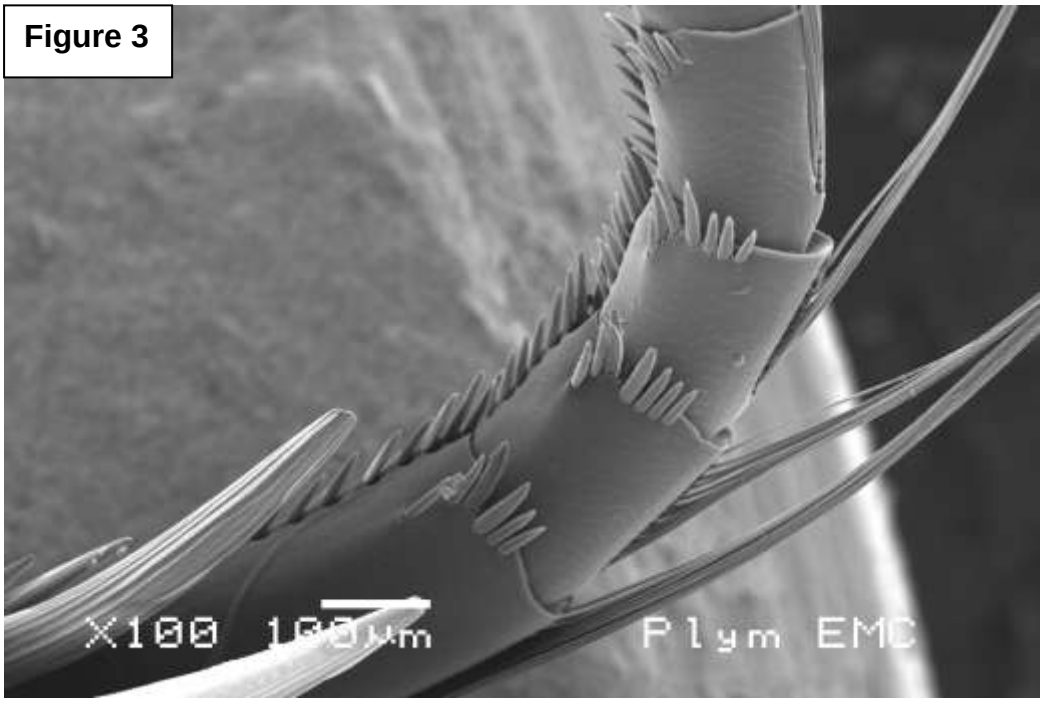
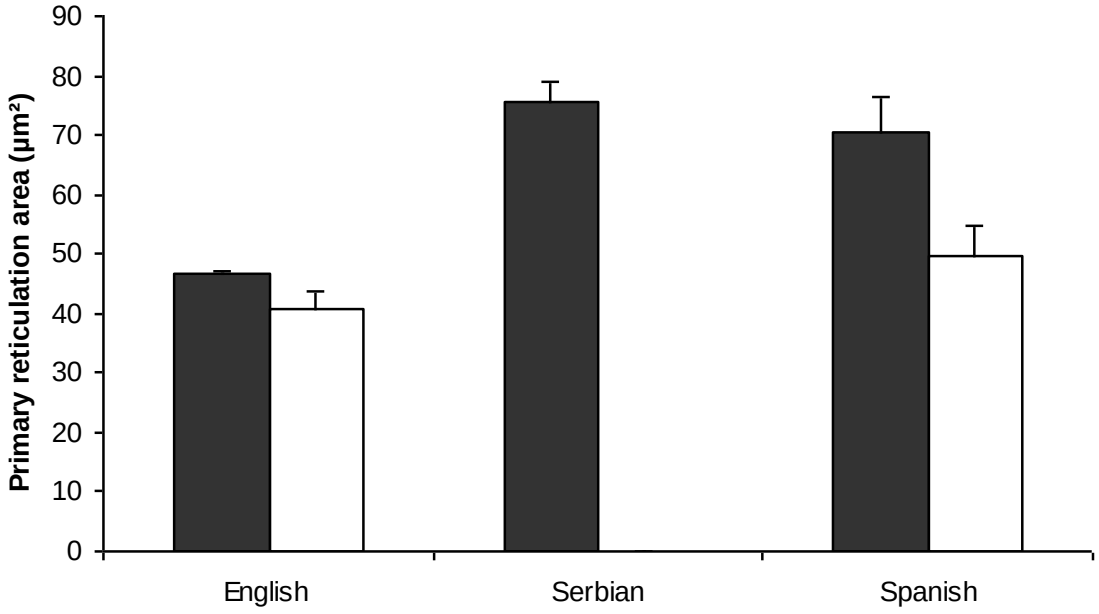
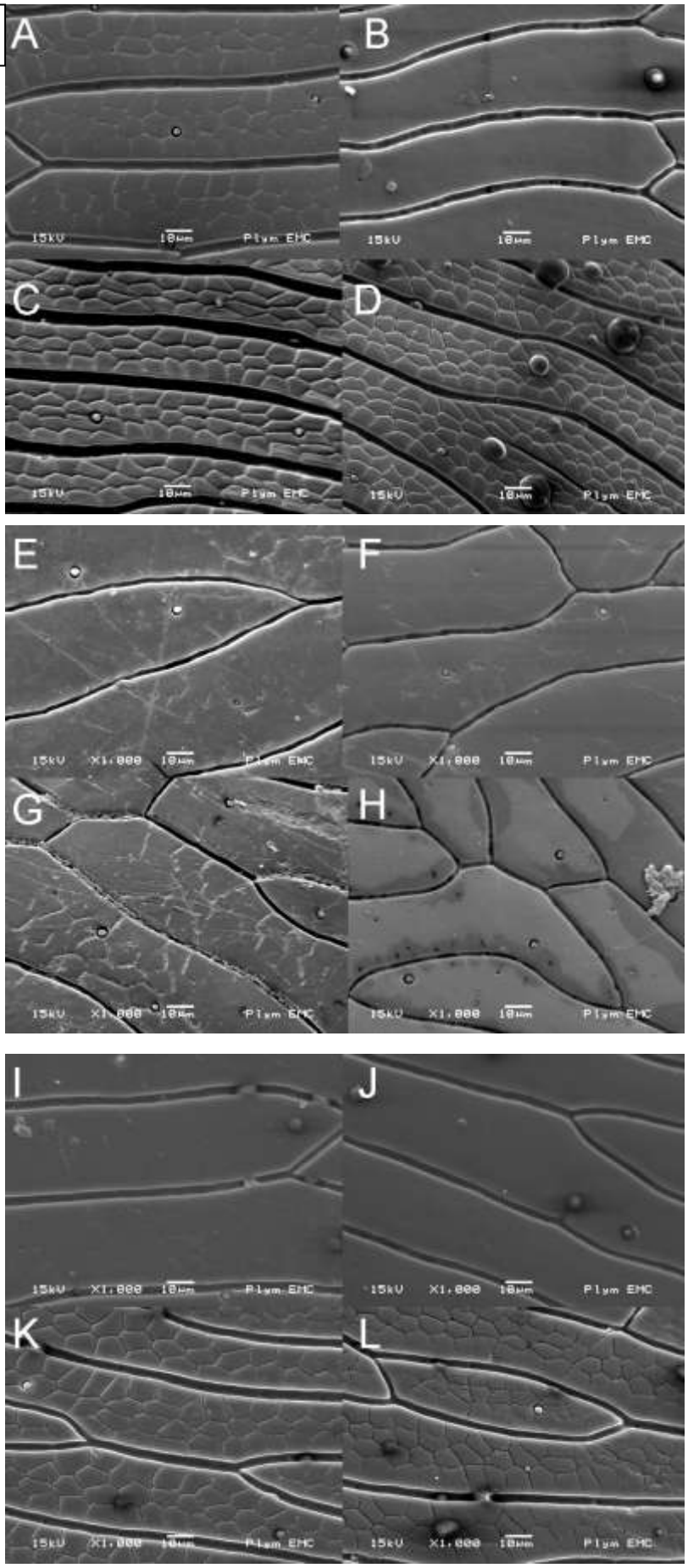


Figure 4



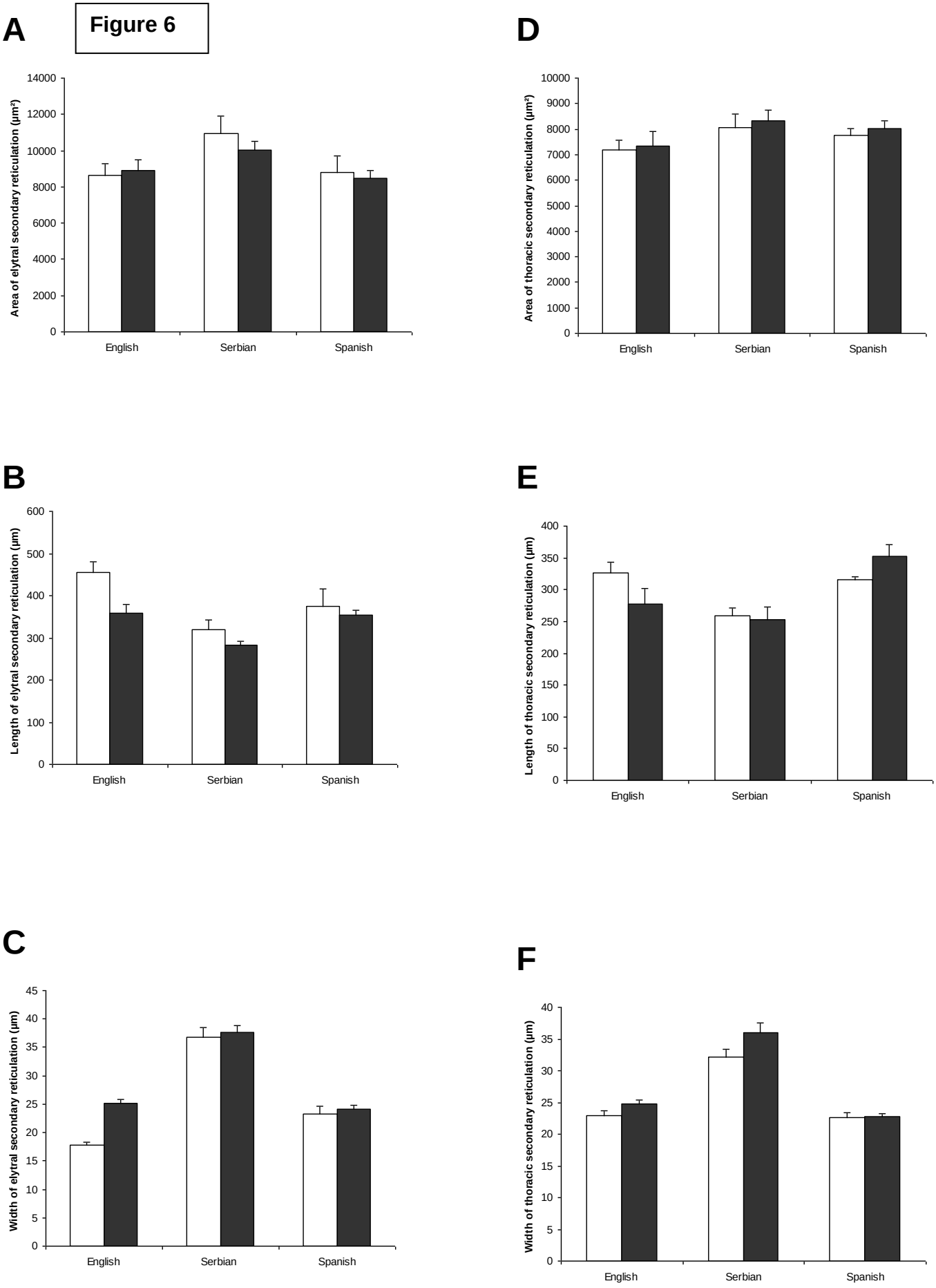
ISSN 1754-2383 [Online]

Figure 5



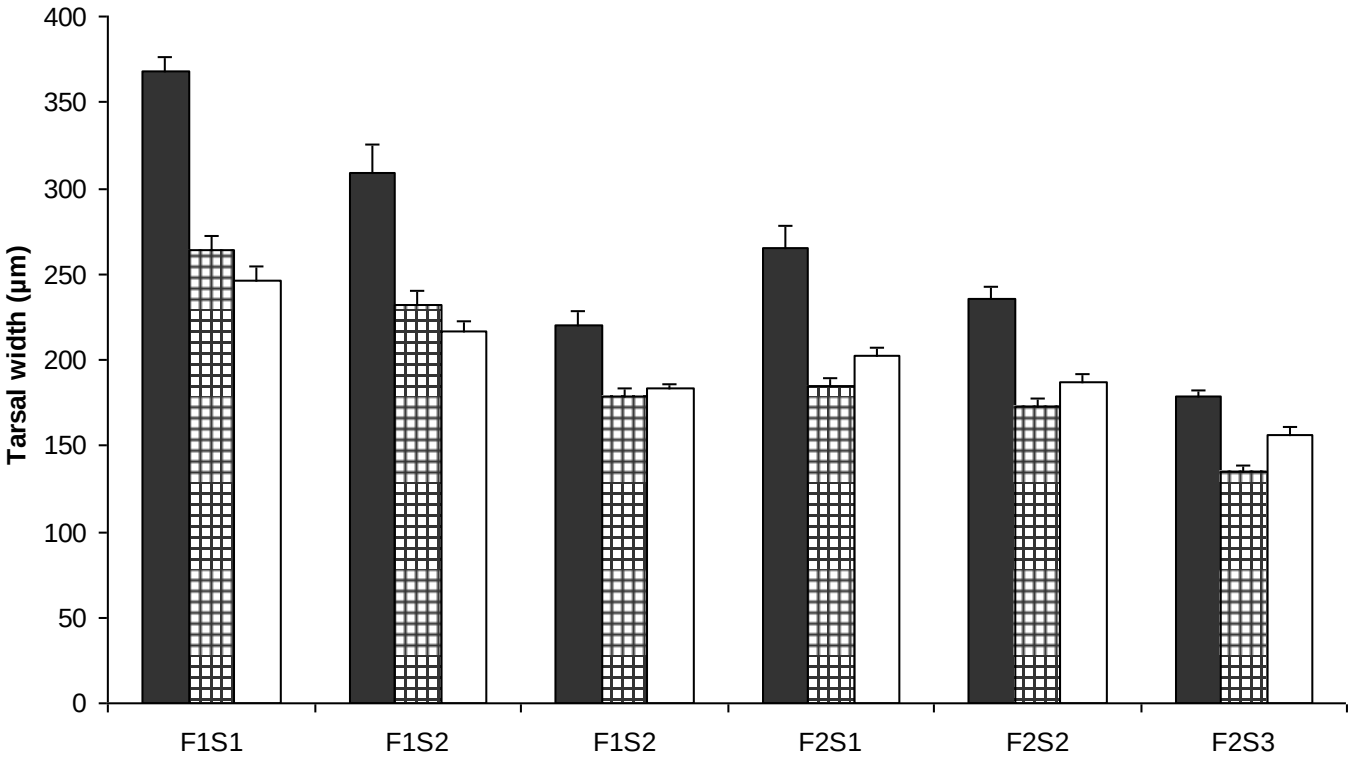
ISSN 1754-2383 [Online]

Figure 6



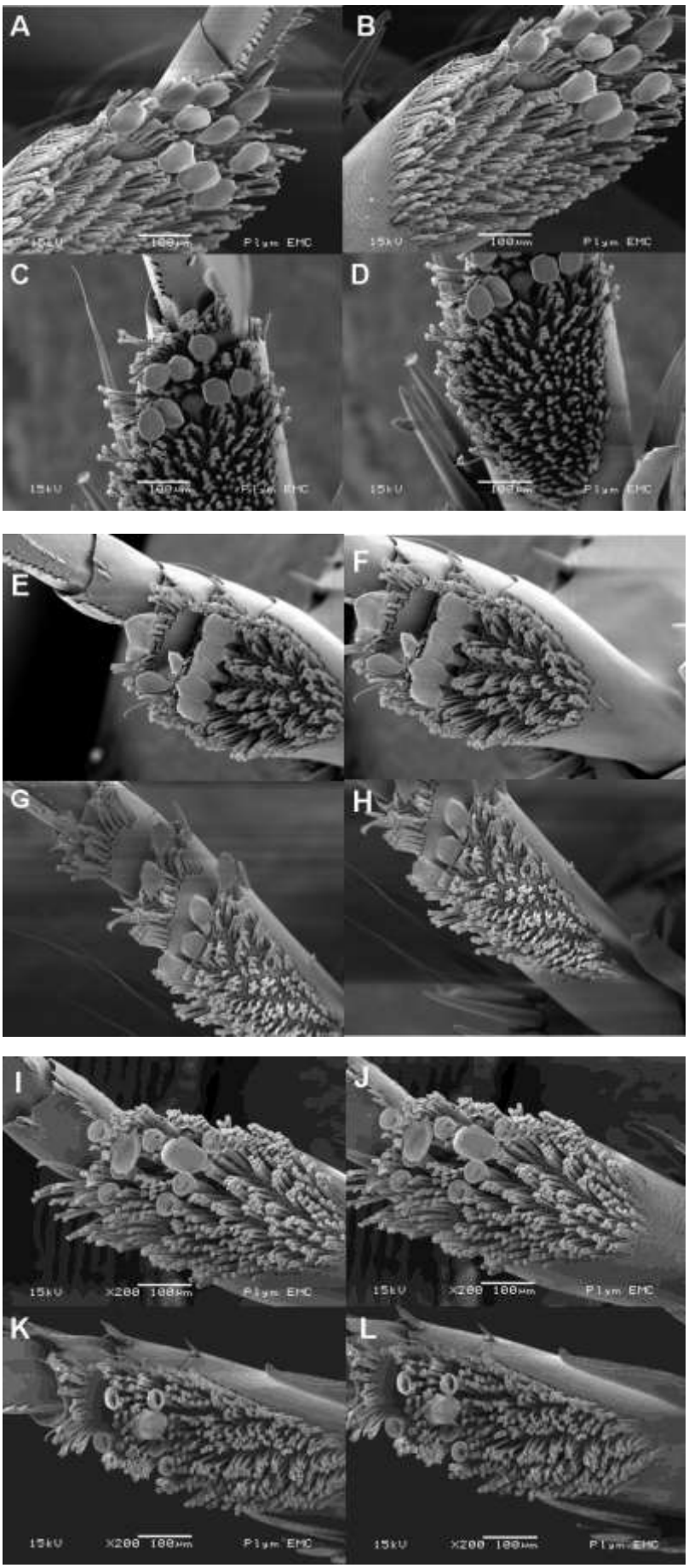
ISSN 1754-2383 [Online]

Figure 7



ISSN 1754-2383 [Online]

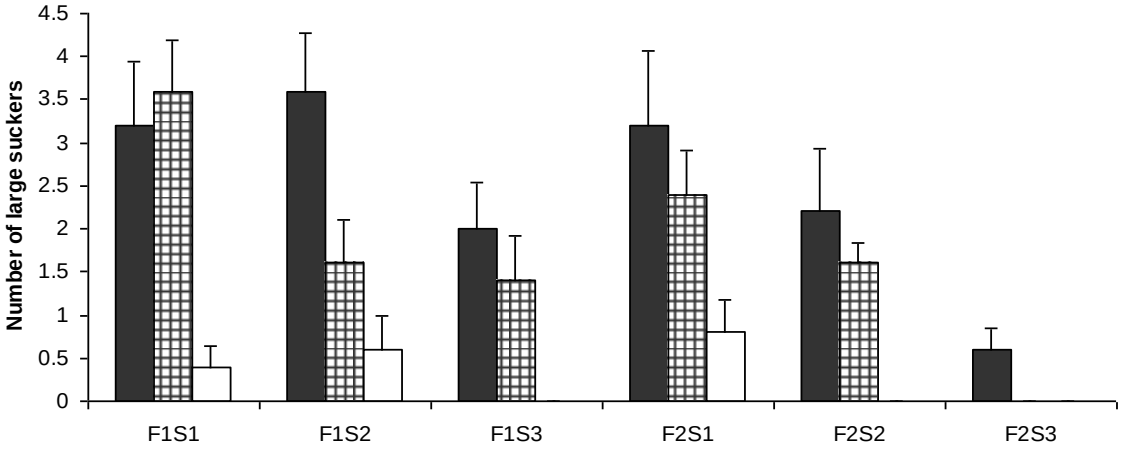
Figure 8



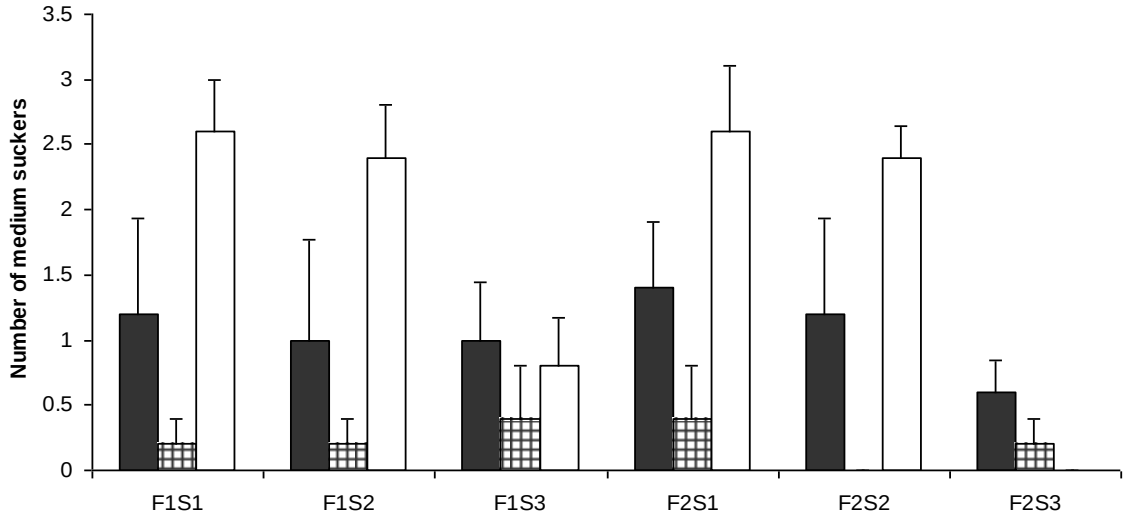
ISSN 1754-2383 [Online]

Figure 9

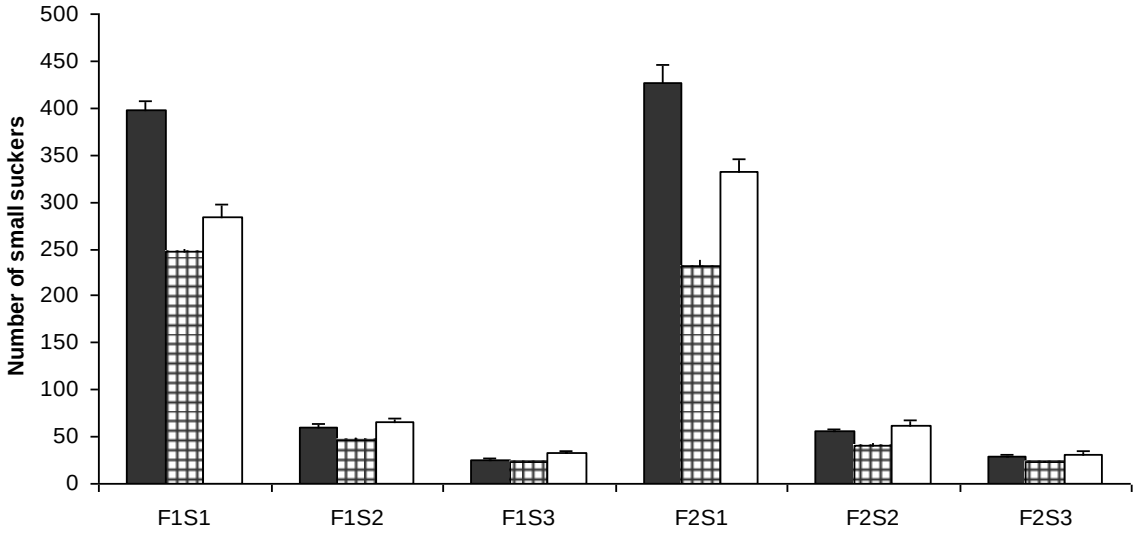
A



B

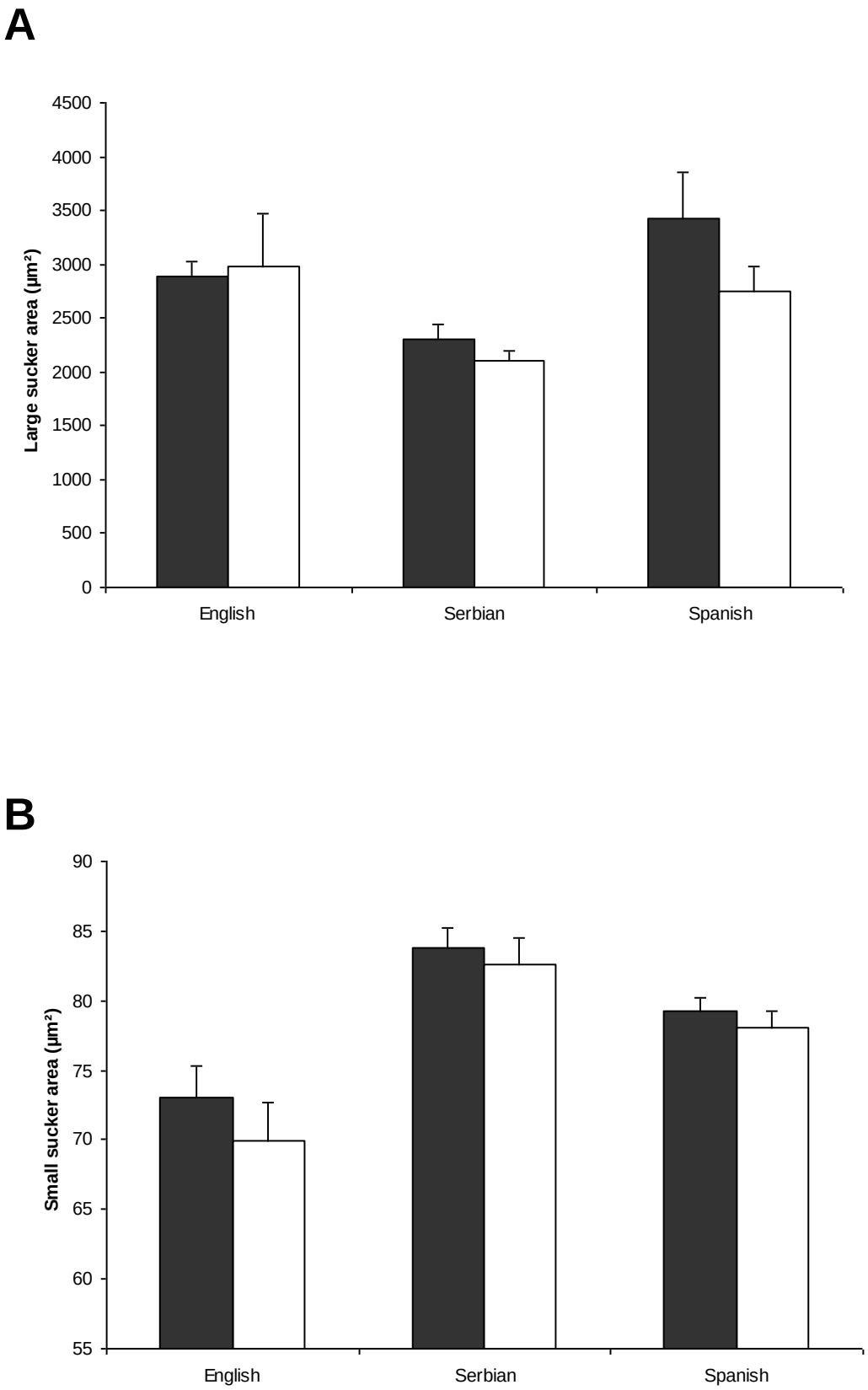


C



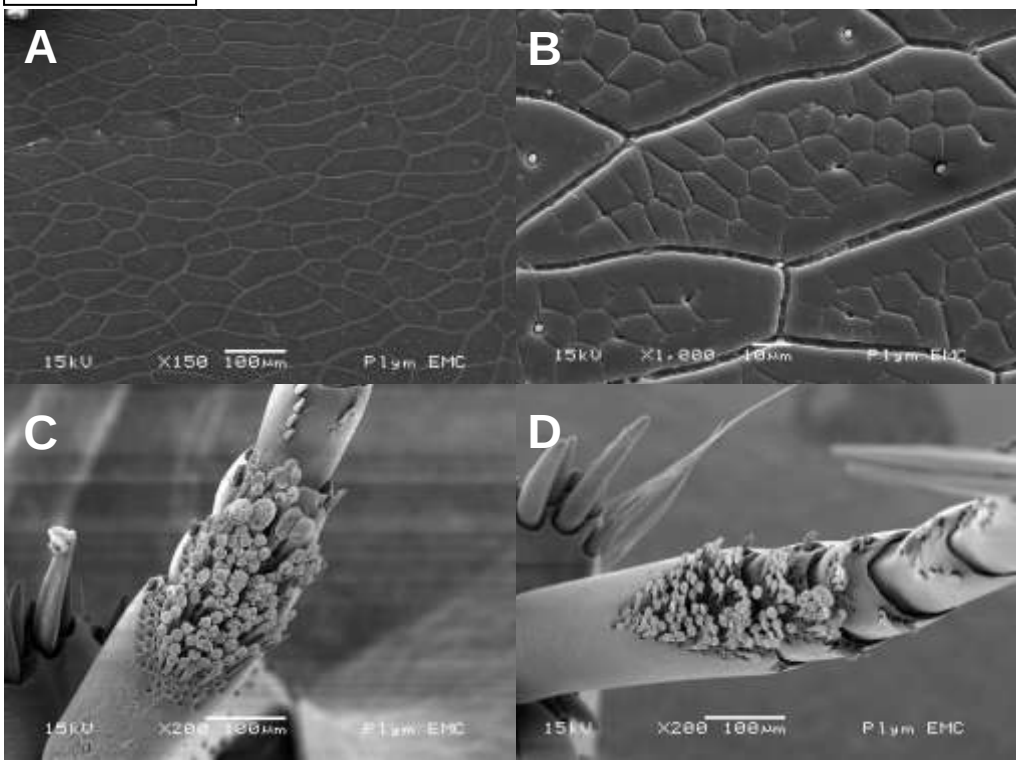
ISSN 1754-2383 [Online]

Figure 10



ISSN 1754-2383 [Online]

Figure 11



ISSN 1754-2383 [Online]

Table 1

Examined specimens of the *Agabus bipustulatus* complex and *Agabus melanarius.*

Population	Locality	Date	Collecter	N♂	N♀
English *(bipustulatus)*	England, Westmorland Rusland pool	9/VI/2007	D.T Bilton	14	11
Serbian *(solieri)*	Kosovo sar planina, Lavadice Lake	10/IX/2007	D.T Bilton	14	16
Spanish (*bipustulatus*)	Madrid, Sierra de Guaderrum	1/VI/2007	D.T Bilton	14	15
A.melanarius	*England, South Devon, Core Hillwood*	26/VIII/2007	D.T Bilton	1	1

Table 2

Percentages of males and females that possess primary reticulation (All collected specimens).

Population	Males	Females **(Elytra)**	Males	Female **(Pronotum)**
English (*Agabus bipustulatus*)	100%	100%	0%	100%
Serbian (*Agabus solieri*)	14%	37.5%	0%	6.25%
Spanish (*Agabus bipustulatus*)	42%	100%	35%	80%

ISSN 1754-2383 [Online]